CLEAN COPY OF CHANGES TO SPECIFICATON

On pg. 2, please amend par. 1 as follows:

Currently, the methods used in the preparation of liposomes, and in the coating of solid liposoluble materials, are well known (Schneider U.S. Pat. No. 4, 089, 801, Ash et al., U.S. Pat No. 4, 448, 765 and Miller et al., U.S. Patent No. 4, 133, 874). The main problems posed by the encapsulation of pharmaceutical materials in liposomes include: the little stability it shows in laboratory trials; the spilling of the encapsulated material; the reduction of the drug's efficacy; the susceptibility shown to adverse environmental conditions, digestion in the gastro-intestinal tract and indirect fusion with cellular (Gregoriadis G, Liposome Technology, Second Edition, Volume II, 1992). On the other hand, liposomes are unstable structures that, for the most part, cannot be freeze dried, a problem which has been overcome by the development of cochlear structures.

On pgs. 2-3, please amend par. 4 as follows:

Cochleates, as well as other auto-assembled micro-structures, have been employed in the administration of therapeutic agents (Yager, et al. U.S. Pat. No. 5, 851, 536, December 22, 1998, Gould-Forgerite, et al. U.S. Pat. No. 5, 994, 318, November 30, 1999 and Yager, et al. U.S. Pat. No. 6, 180, 114, January 30, 2001). These include preparations of cochleates containing adjuvants (Gould-Fogerite et al. U. S. Pat. No 5, 994, 318, November 30, 1999). Nevertheless,

both liposomes and cochleates must be derived from negative lipids in the presence of cholesterol, both of which are generally extracted from animals in costly procedures (Mannino et al. U.S. Pat. No. 4,663,161. May 5, 1987) that are increasingly less acceptable in light of new pharmaceutical regulations and that preferably include a purified protein obtained from a microorganism, or a peptide, in the case of vaccine preparations. Furthermore, we should point out that the use of cochleates as adjuvants in their own right, or their preparation with other activators of important signals in the inducement of immunological responses, such as pathogens-associated molecular pattern (phylogenetically preserved structures for which there are receptors in the host (pathogens recognition receptors), and which are recognized as a sign of danger by that host), has not been previously considered.

On pg. 3, please amend pars. 1 and 2 as follows:

The aim of the present invention is to obtain a new cochlear structure from outer membranes vesicles of live organisms, which present adjuvant and vaccinal properties due to their particular protein and lipidic make-up, as well as to the pathogens-associated molecular pattern found in the organism. Once formed, the cochlear structures are homogenized at their own size to make them more immunologically effective.

The cochlear structures obtained through the present invention are characterized by the fact that, having a proteolipidic composition as yet untried by other authors, they are capable of auto-assembling themselves and of producing rolled, sea-shell shaped structures. The protein and lipidic compositions of the cochlear structures will depend on the microorganism that has supplied its outer membrane vesicles, that is, it will depend on the characteristics of the proteins found in its membrane. In the same fashion, the mentioned structures contain concentrations of pathogens-associated molecular pattern, between 1 and 7 % in relation to the concentration of proteins, supplied by the membrane of the microorganism in question, which are inserted and are not found within it in a free state. Furthermore, these structures can be purified from other microorganisms and can be added to the preparation. The added and existing structures must be at a concentration between 1 and 30 % in relation to the concentration of proteins. One of the pathogens-associated molecular pattern utilized during the production of cochlear structures was the lipopolysaccharide of Vibrio cholerae or N. meningitidis (example 20).

On pg. 4, please amend par. 2 as follows:

With respect to the vaccine composition containing said structure, it is important to point out that the response of the serums was superior, at various times of the experiment, to that obtained while employing an effective vaccine based on outer membrane vesicles with an aluminum hydroxide adjuvant, known in the market as VA-MENGOC-BC™, (Sierra GVG et al. 1991. NIPH Annals. 14:195-210). and that it also induced immunoglobulin A specific on being administered through the mucosa. Furthermore, the cochlear structures stimulated CD8 lymphocytes, an important part of all immunological responses to intra-cellular organisms.

On pg. 4, please amend pars. 3-5 as follows:

The adjuvant action of the cochlear structures was evaluated through various tests, including: the production of IL12 in the human histiocytary cell line U937 (example 11) and the production of nitric oxide in the murine macrophagyc cell line J774, in the absence of all stimuli (example 13); the stimulation of human dendrites (example 15) and the reduction in the induration of lesions in the challenge experiment, "challenging" mice immunized with cochlear structures containing antigens derived from the protozoan organism with Leishmania major (example 16).

The cochlear structures produced through the invention make the resulting adjuvant or vaccine induce earlier, stronger and more durable responses "in vivo", while an efficient induction of mediators involved in the induction of a cellular response, and a good stimulation of cells presenting professional antigens (dendritic cells), was observed "in vitro" (examples 11, 13, and 15).

Another aim of the present invention is the use of outer membrane vesicles of microorganisms, which constitute the starting point for the formation of cochlear structures, as vaccines or as heterologous adjuvants, such that eliminating the adsorption of aluminum hydroxide does not limit the immunogenic capabilities of the former. It is worth mentioning that the capacity of these vesicles to induce parenteral responses all by themselves has not been sufficiently investigated.

On pg. 5, please amend pars. 1-3 as follows:

Said vesicles are known as auto-assembled nanospheres, and are constituted by a lipidic bi-layer with proteins and polysaccharides inserted in it. These can be extracted from any pathogen and may present different molecular structures (especially lipopolysaccharide, peptidoglycan, lipoprotein, teicoic acid, flagellin or lipophosphoglycan). Lipophosphoglycan and lipopolysaccharide were obtained from Leishmania major and N. meningitidis or salmonella typhl,

respectively, during the process of obtaining the outer membranes vesicles of the organisms, remaining inserted in the former and never in a free state, in proportions between 1 and 7 % of the protein weight.

Used in the vaccine preparations, the outer membrane vesicles extracted from salmonella typhl or from N. meningitidis B induced a response from the IgA when inoculated nasally, and a good immunological response when parenterally inoculated (examples 3, 5, 7, 12 and 14).

The adjuvant effect of the outer membrane vesicles was evaluated through a number of tests, including: the production of IL12 in the human histiocytic line U937 (example 12) and the production of nitric oxide in the murine macrophagic line J774 in the absence of other stimuli (example 14); the strengthening of the cellular response (an increase of IgG2a) through the combination of polysaccharides with outer membrane vesicles of N. meningitides, in comparison to its fusion with a tetanus toxoid (example 18) and the strengthening of the response of antibodies reactive against polysaccharides Vi from S. typhl through combination with outer membrane vesicles found in the same bacteria (example 19).

On pgs. 5-6, please amend par. 5 as follows:

The invention also discloses a method for obtaining cochlear structures from the outer membranes vesicles of live organisms. The following steps are taken to achieve this: In the first place, the outer membrane vesicles of live microorganisms or cells, is purified using any of the methods widely employed by experts in the field. The preferred methods are those disclosed in EP 301992, US 5,597,572, EP 11243 or US 4,271,147, Zollinger et al. (J. Clin. Invest. 1979, 63:836-848), Frederikson et al. (NIPH Annals 1991, 14: 67-80), Sauders et al. (Infect. Immun. 1999,67:113-119), Drabick et al. (Vaccine 2000, 18:160-172), WO 01/09350 or EP 885900077.8 and US 5,597,572. The membranes are so purified that they contain between 1 and 7 % of lipopolysaccharide, completely inserted into the vesicle. A solution of a total protein concentration between 3 and 6 mg/mL is prepared, increasing the concentration of non-ionic detergent to 8 to 12 times that of the protein concentration, in order to completely dissolve the vesicles. This solution is subsequently sterilized by filtration through a membrane with a pore size of 0.2 μm, in which the vesicle aggregates which had not been dissolved are also eliminated. Following this, a rotational dialysis or tangential filtration is carried out. The dialysis is carried out for 24 hours against a solution containing adequate concentrations of a multivalent ion (particularly Ca^{2+}, Zn^{2+} or Mg^{2+}, at concentrations ranging from 2.5 to 6.5 mM) at a pH condition of pH 7.4 ± 0.2. Finally, the cochlear structures obtained are submitted to a mechanical

treatment (sonication in a water bath between 15 °C and 25 °C in temperature for 45 minutes, in particular), in order to homogenize the particle sizes.

On pg. 6, please amend par. 1 as follows:

This constitutes a rapid and efficient method for obtaining cochlear structures which contain multiple proteins and lipids from the outer membrane of the microorganism employed, as well as pathogens-associated molecular pattern naturally obtained. These structures demonstrate a high level of stability and immunogenicity.

On pgs. 6-7, please amend par. 2 as follows:

On the other hand, the uncomplicated and efficient method for obtaining them permits us to introduce new antigens to the mentioned structure. The new antigens are added to the suspension of outer membrane vesicles prepared for obtaining said structures, after increasing the concentration of detergents and prior to the addition of the multivalent ions during the process of dialysis. Among the antigens that may be added are the saccharides, lipoproteins, peptides, conjugates and nucleic acids. These must be at a concentration between 0.2 to 2.7 μg for every 3 to 9 μg of proteins. It is also possible to incorporate other pathogens-associated molecular pattern to stimulate the innate and acquired response, something which renders it useful as heterologous adjuvants. The lipopolysaccharide of vibrio cholerae, amastigotes or promastigotes of

Leishmania mayor, were the structures especially employed, which allowed us to induce cellular responses as well as the activity of antibodies reactive against them. In addition to this, plasmidic DNA, containing fluorescent-green protein, was introduced and placed against macrophagic lines; the molecule's fluorescence later allowed us to determine its intracellular presence. An allergen derived from dermatophagoides siboney was also introduced and the resulting cellular response induced was determined (examples 16 and 17).

On pg. 7, please amend Example 1. as follows:

Example 1. Obtaining the cochlear structures

On pg. 7, please amend par. 1 as follows:

The use of live organisms as a source of raw material for obtaining cochlear structures has not been described by any author. Neither has the process of incorporating one or more pathogens-associated molecular pattern into the cochlear structures, as in the case of the present invention.

On pg. 8, please amend par. 1 as follows:

The concentration of proteins and phospholipids was once again calculated and adjusted for subsequent trials. The physical and chemical properties of the proteins included in the cochlear structures were checked and compared with that of the vesicles through electrophoresis in polyacrylamide gels stained with Coomassie Blue. The structural integrity of the latter was determined and confirmed using the Western Blot method (Fig. 2 – 4).

On pg. 8, please amend par. 2 as follows:

Balb/c mice were intra-muscularly immunized with 12 μg of proteins per mice, in 2 doses separated by 21 days, with VA-MENGOC-BC® or cochlear structures. Blood samples were taken from the animals at the indicated times following the second dose and the IgG responses in the sera against outer membrane vesicles were evaluated through an ELISA test. Significant differences ($p<0.05$) were observed between the responses induced by the cochlear structures and the vaccine, always in favor of the former, at 17, 27 and 180 days following the second dose (Fig. 5)

On pg. 8, please amend par. 3 as follows:

Balb/c mice were intra-muscularly immunized with 12 μg of proteins per mice in two doses separated by 21 days, with VA-MENGOC-BC® or outer membrane vesicles. Blood samples were taken from the animals at the indicated times following the second dose and the IgG responses in the sera against the vesicles were evaluated through an ELISA test. No significant differences ($p<0.05$) were observed between the responses induced by the vesicles and those induced by the vaccine. These results confirm the usefulness of the vesicles and vaccines in their own right (Fig. 6)

On pgs. 8-9, please amend par 4 as follows:

Balb/c mice were intra-nasally (IN) or gastrically (IG) immunized with 100 or 12 μg of proteins per mice, in two doses separated by 21 days, respectively. Blood samples were taken from the animals at the indicated times after the second dose and the sera responses of IgG against the vesicles were evaluated through an ELISA test. Good responses from the IgG were induced against the outer membrane vesicles with both concentrations of cochlear structures intra-nasally and gastrically inoculated. This suggests that good systemic responses are to be gotten through mucosal inoculation (Fig. 7).

On pg. 9, please amend par. 1 as follows:

Balb/c mice were intra-nasally (IN) immunized with 12 μg of proteins per mice, in two doses separated by 21 days. Blood samples were taken from the animals at the indicated times after the second dose and the IgG responses in the sera against the vesicles were evaluated through an ELISA test. Good IgG responses against the vesicles were obtained through this inoculation method, suggesting that valuable systemic responses can be obtained through intra-nasal inoculation (Fig. 8)

Please amend Example 6. as follows:

Example 6. Effectiveness of intra-nasally or gastrically administered cochlear structures in inducing IgA in saliva

On pg. 9, please amend par. 2 as follows:

Balb/c mice were intra-nasally (IN) or gastrically (IG) immunized with 100 or 12 μg of proteins per mice, in two doses separated by 21 days, respectively. Saliva samples were taken from the animals 9 days after the last dose was administered and the response of IgA against outer membrane vesicles was evaluated through an ELISA test. Significant responses of IgA against the vesicles were obtained using the IN method and a small but important increase in the IgA anti-vesicles was obtained using the IG method (Fig. 9).

On pg.10, please amend Example 8. as follows:

Example 8. Subclasses of IgG reactive against outer membranes vesicles in serum induced through immunization with cochlear structures

On pg. 10, please amend par. 1 as follows:

Balb/c mice were intra-nasally (IN), -gastrically (IG) or -muscularly (IM) immunized. In the case of the IN method, a concentration of 100 μg of proteins per mice of the cochlear structures was administered, while a concentration of 12 μg was used in the rest of the cases. The doses were separated by a period of 21 days in all cases. The vaccine VA-MENGOC-BC® was employed as a positive control, being intra-muscularly administered at a concentration of 12 μg.

Blood samples were taken from the animals 21 days after the second dose was administered and the titers of IgG1 and IgG2a present in the serum were determined through an ELISA test. In all of the cases considered (with the exception of the negative control cases), significant titers for IgG2a were obtained ($p<0.05$). These were at their highest value when the cochlear structures were administered intra-nasally. This indicates the inducement of a pattern of IgG, cellular Th1 type antibodies, especially favored by nasal inoculation (Fig. 11).

On pg. 10, please amend par. 2 as follows:

Balb/c mice were intra-nasally (IN) and -muscularly (IM) immunized with 12 μg of proteins per mice of outer membrane vesicles, in two doses separated by 21 days. The vaccine VA-MENGOC-BC® was administered at the same concentration as a positive control. Blood samples were taken 21 days after the second dose was administered and the titers of IgG1 and IgG2a anti OMV present in the serum were analyzed through an ELISA test. In all of the cases considered, significant titers of IgG2a were induced by the outer membrane vesicles, indicating the inducement of a pattern of cellular Th1 type IgG antibodies. This was not the case with the negative IM or IN controls. A complete reversal of the pattern was observed in the case of IN inoculation, where the response was almost exclusively that of IgG2a (Fig. 12).

On pg. 10, please amend Example 10 as follows:

Example 10. Thermal and acidic resistance of the cochlear structures obtained from outer membrane vesicles

On pg. 11, please amend pars. 2-3 as follows:

U937 cells were cultivated in RPMI 1640 supplemented with gentamicin at a concentration of 50 μg/mL, L-glutamine (at 2 mM), sodium pyruvate (at 1mM), HEPES (at 15 mM) and fetal bovine serum (Sigma) at 10 %. These were differentiated into macrophages through a PMA treatment and were placed in flat-bottomed, 24-hole culture dishes, 5 x 10^5 cells per hole. After 24 hours, the cochlear structures were added to them at a concentration of 250 ng/mL in the culture medium. After 24 hours of stimulus, the surviving cells were gathered and the presence of IL12 was determined through a sandwich-type ELISA test. The production of IL12 by the U937 cells stimulated by the cochlear structures was observed (Fig. 14).

U937 cells were cultivated in RPMI 1640 supplemented with gentamicin at a concentration of 50 μg/mL, L-glutamine (at 2 mM), sodium pyruvate (at 1mM), HEPES (at 15 mM) and fetal bovine serum (Sigma) at 10 %. These were differentiated into macrophages through a PMA treatment and were placed in flat-bottomed, 24-hole culture dishes, 5 x 10^5 cells per hole. After 24 hours, the outer membrane vesicles were added to them at a concentration of 250 ng/mL in

the culture medium. After 24 hours of stimulus, the surviving cells were gathered and the presence of IL12 was determined through a sandwich-type ELISA test. The production of IL12 by the U937 cells stimulated by the outer membrane vesicles was observed (Fig. 15).

On pg. 12, please amend pars. 1 and 2 as follows:

J774 cells were cultivated in a D-MEN medium supplemented with gentamicin at a concentration of 50 μg/mL, L-glutamine (at 2 mM), sodium pyruvate (at 1mM), HEPES (at 15 mM) and fetal bovine serum (Sigma) at 10 %, previously inactivated at 56° C for 30 minutes. They were placed in flat-bottomed, 96-hole culture dishes, at a concentration of 1 x 10^5 cells per hole and they were incubated for a period of 24 hours at 37°C and 5 % CO2. Following this, the adhering cells were incubated with μ-L of D-MEN along with the cochlear structures at a concentration of 250 ng/mL. Other variants incubated with L-NMMA (at 1 μM), an inhibitor of the production of nitric oxide, were also included. The surviving cells were collected after 24 and 48 hours and analyzed for nitric contents using Greiss' reaction (Rockett, KA et al., Infect. Immun. 1992, 60:3725-3730). A significant production of nitric oxide by the cells incubated with the cochlear structures was observed. This production was inhibited by the use of L-NMMA (Fig. 16).

J774 cells were cultivated in a D-MEN medium supplemented with gentamicin at a concentration of 50 μg/mL, L-glutamine (at 2 mM), sodium pyruvate (at 1mM), HEPES (at 15 mM) and fetal bovine serum (Sigma) at 10%, previously inactivated at 56 °C for 30 minutes. They were placed in flat-bottomed, 96-hole culture dishes, at a concentration of 1 x 10^5 cells per hole and they were incubated for a period of 24 hours at 37°C and 5 % CO2. Following this, the adhering cells were incubated with 200 μ-L of D-MEN along with the outer membrane vesicles at a concentration of 250 ng/mL. Other variants incubated with L-NMMA (at 1 μM), an inhibitor of the production of nitric oxide, were also included. The surviving cells were collected after 24 and 48 hours and analyzed for nitric contents using Greiss' reaction (Rockett, KA et al., Infect. Immun. 1992, 60:3725-3730). A significant production of nitric oxide by the cells incubated with the outer membrane vesicles was observed, greater than that induced by the LPS utilized as a control. This production was inhibited by the use of L-NMMA.

On pg. 13, please amend Example 15. as follows:

Example 15. Stimulation of human dendrytic cells by the cochlear structures

On pg. 13, please amend Example 16. as follows:

Example 16. Reduction of indurations in Balb/c immunized with cochlear structures containing amastigotes of Leishmania major and challenged with the same protozoan organism

On pg. 13, please amend par. 2 as follows:

The inclusion of amastigotes derived from L. major was achieved by including the semi-purified antigens in the first steps of the formation of the cochlear structures. The amount of detergent used was adjusted to the total protein content and the total concentration of proteins was maintained at a range of 5-6 mg/mL. The ratio of vesicular proteins to the new antigens included was that of 12:1. The formation of cochlear structures was verified through optical and electronic microscopy. The inclusion of proteins from L. major was also verified through electrophoresis in polyacrilamyde gels stained with Coomassie Blue. Balb/c mice were intra-muscularly immunized with 12 μg of the cochlear structures in 2 doses separated by 21 days. The cochlear structures were inoculated at the left posterior extremity. After 21 days following the second dose, the mice were infected with 3×10^6 promastigotes at the same extremity inoculated. The promastigotes were obtained from the stationary phase of the cultures grown in a D-MEN medium over a solid agar-blood medium. The volume of lesions was stimulated weekly starting at the fourth week following the infection. A significant reduction in the size of the lesions was observed in the group immunized with the cochlear structures containing antigens of L. major. This demonstrates the adjuvant character of this structure (Fig. 19).

On pg. 14, please amend par 1 as follows:

Purified plasmids containing the gene of the fluorescent green protein under a CMV promoter were included in the initial solution used for obtaining the cochlear structures, following the same steps for obtaining said structures described in Example 1. The ratio of plasmids to vesicular proteins was adjusted to 1:100. The inclusion of the plasmids was checked using electrophoresis in agar gel at a 1% concentration of the cochlear structures previously incubated at 37°C for 30 minutes, after adding EDTA to a quantity of 2 mM in order to provoke the freeing of plasmids inside them. The gels were stained with ethidium and were observed under ultraviolet light. The presence of plasmids was detected only in the cochlear structures that contained them after being treated with EDTA. Following this, a transfection trial was carried out in the J774 cell line using these structures. After 2 hours of incubation, the cochlear structures were eliminated from the culture medium. The inspection of the cells under fluorescence 24 hours later revealed the presence of numerous cells with fluorescent signals in the cytoplasm.

On pg. 14, please amend Example 18. as follows:

Example 18. Strengthening of cellular response through the conjugation of outer membrane vesicles

On pg. 14, please amend par. 3 as follows:

The polysaccharide Vi from salmonella typhl was conjugated to outer membrane vesicles (OMV) of S. typhl Balb/c mice were intra-peritoneously immunized with two doses (administered at day 0 and 28) containing 10 μg of the Vi. Blood samples were taken from the animals before and 42 days following the inoculation. The responses of IgG and its subclasses in the serum were determined. Conjugation increases and becomes in positive to the response against the Vi polysaccharide and a response offered by IgG2a is detected (Fig. 21).

On pg. 15, please Example 20 as follows:

Example 20. The possibility of including different concentrations of pathogens-associated molecular pattern in cochlear structures

On pg. 15, please amend par. 1 as follows:

Different quantities of LPS derived from neisseria meningitidis B were experimented with for the inclusion of different concentrations of pathogens-associated molecular pattern in the cochlear structures. The ratios of LPS concentration to protein concentration used for the immunization of the mice were: 0.05:12, 0.5:12, 1:12, and 2:12. The formation of cochlear structures was verified through optical microscopy, which determined a ratio of 1:12 as the maximum ratio in which the LPS may be introduced without affecting the

formation of the cochlear structures. Larger quantities visibly affect the formation of the structures, resulting in the formation of aggregates. All of the obtained variants were administered to Balb/c mice in two doses, separated by 21 days, of 12 μg of proteins per mice. The titers of anti-vesicle IgG were determined. No difference between the titers resulting from the use of the different variants were observed. The experiment guarantees the possibility of incorporating different LPS in these structures, however (Fig. 22).

On pg. 16, please amend par. 2 as follows:

Balb/c mice were intra-nasally (IN) immunized with 2 or 3 doses of outer membrane vesicles (OMV), muscularly administered 3 doses of the vaccine VA-MENGOC-BC® as a control, and a combination of 1 and 2 doses of the vaccines administered using the IM and IN methods, respectively. Each mouse was administered 12 μg of protein at times 0, 21 and 42. The serums were taken after 15 days and the saliva and vaginal fluid after 9 days following the last dose. The results were evaluated through an ELISA test. As can be observed, nasal immunization induces a small increase of the IgA at the level of the serum, while the immunization with the vaccine using the IM method does not. The mucosal response depended on the number of doses: two doses did not induce a response, while 3 doses resulted in a response of anti-vesicle IgA. Finally, 2 doses administered nasally proved effective in animals that received a stimulus (one dose) of the vaccine administered intra-muscularly (Fig. 24).

On pgs. 17-18, please amend sections 2-3,5,8,9,11 and 12 as follows:

2. during the extraction process, other proteins of interest, be they natural or re-combining, may be included;
3. outer membrane vesicles extracted from live organisms are more stable than artificially constructed liposomes, and can remain intact for a number of months, even years, without suffering significant alterations that can affect the formation of future cochlear structures;

5. the cochlear structures formed are thermo-resistant, something which can prove useful in solving the problems associated with the chain of cold of a number of vaccines, be it through their formulation as an adjuvant or their development from outer membrane vesicles and cochlear structures;

8. the versatility of the antigens that may be included, be they soluble or particulated, including nucleic acids, allows for the production of a great many vaccines, including multiple ones;

9. the cochlear structures contain pathogens-associated molecular pattern, and others may be incorporated at will in order to increase its adjuvant and immunological effectiveness, allowing us to reduce the potential toxicity of some of these structures and thus their inflammatory effects;

11. the cochlear structures induce, in vitro, better responses at the level of cytokines which induce patterns of cellular immune responses;

12. the structures preserve the properties of artificial cochleates (the efficient incorporation of hydrophobic antigens, slow deployment system, the content of calcium as an essential mineral, the reduction of lipidic oxidation, freeze drying, etc.), but it is superior to these in immunogencity, its inclusion of pathogens-associated molecular pattern and in its capacity to induce a Th1

pattern, including a T cytotoxic response and the use of lipids and cholesterol, derived from animal serum, is avoided.

On pgs. 18-19, please amend figures 4, 7-8, 19,21-22, 25-29 as follows:

Figure 4. A: Electrophoresis in acrylamide gel at 12.5 % tinted with Coomassie Blue of the proteins present in the outer membrane vesicles. B: Western Blot of the proteins present in outer membrane vesicles and the cochlear structures, using a human serum of a high titer of antibodies reactive against outer membrane vesicles.

Figure 7. Serum responses of IgG against outer membrane vesicles in mice intra-gastrically (IG) or -nasally (IN) immunized with outer membrane vesicles, evaluated by an ELISA test.

Figure 8. Serum responses of IgG against outer membrane vesicles in mice intra- intra-nasally (IN) immunized with outer membrane vesicles, evaluated by an ELISA test.

Figure 19. Results of the indurations in animals immunized with cochlear structures containing amastigotes and challenged with Leishmania major.

Figure 21. Results of the adjuvant effect of the conjugation of polysaccharide with outer membrane vesicles of salmonella typhl.

Figure 22. Results of the incorporation of pathogens-associated molecular pattern.

Figure 25. Results of the kinetic of IgG anti-OMV response potenciates by the cochleate structure (AFCo1).

Figure 26. Results showing that DC can process Ova peptides from Ova included in the outer membrane vesicles (OMV) (OMV-Ova) for MHC-II presentations.

Figure 27. Results showing the IgG anti-Ova response in mice immunized with OMV-Ova.

Figure 28. Results showing the IgG anti-Ova subclasses response in mice immunized with OMV-Ova.

Figure 29. Results showing the IgG response against Core protein of the Hepatitis C Virus (VHC) induced by intramuscular immunization of animals.